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(54) Title: ISO-CYCLOSPORIN SALTS

(57) Abstract

Acid addition salts of iso-cyclosporins are found to have surprisingly improved galenical characteristics, e.g., enhanced solubility and stability, and to demonstrate an improved pharmacologic profile compared to cyclosporins, making them useful as pharmaceuticals, e.g., as immunosuppressants, particularly as pro-drugs for cyclosporins. Novel iso-cyclosporins, which are useful, e.g., for making the iso-cyclosporin acid addition salts of the invention, are also provided, as are methods of production, pharmaceutical formulations, and therapeutic applications for the compounds of the invention.

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ISO-CYCLOSPORIN SALTS

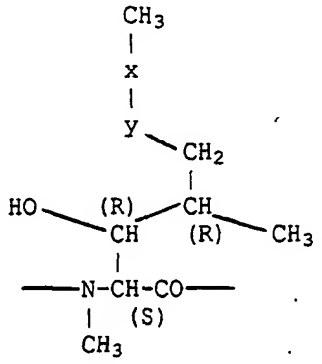
The present invention relates to novel iso-cyclosporin acid addition salts and iso-cyclosporins, their use as pharmaceuticals and pharmaceutical compositions comprising them, as well as to processes for their production.

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological, in particular immunosuppressive, anti-inflammatory and/or anti-parasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite Ciclosporin or Cyclosporine, also known as cyclosporin A and commercially available under the Registered Trademark SANDIMMUN^R or SANDIMMUNE^R. Ciclosporin is the cyclosporin of formula I

MeBmt-	αAbu-	Sar-	MeLeu-	Val-	MeLeu-	Ala-	(D)Ala-	MeLeu-	MeLeu-	MeVal
1	2	3	4	5	6	7	8	9	10	11

(I)

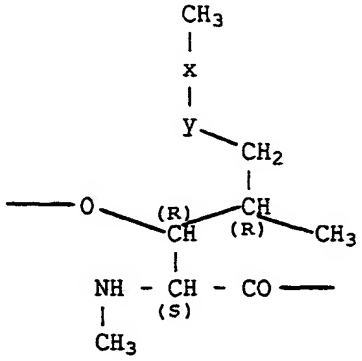
wherein MeBmt represents the N-methyl-(4R)-4-but-2E-en-1-yl-4-methyl-(L)threonyl residue of formula II



(II)

in which -x-y- is -CH=CH- (trans).

Since the original discovery of Ciclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprised by the cyclosporins is thus now substantial and includes, for example, the naturally occurring cyclosporins A through Z [cf. Traber et al, 1, Helv. Chim. Acta, 60, 1247-1255 (1977; Traber et al, 2, Helv Chim. Acta 65 1655-1667 (1982); Kobel et al, Europ J Applied Microbiology and Biotechnology 14 273-240 (1982); and von Wartburg et al, Progress in Allergy 38 28-45 (1986)], as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporins including the dihydro-cyclosporins, in which the moiety -x-y- of the -MeBmt- residue (formula II above) is saturated to give -x-y- = $-CH_2-CH_2-$ and iso-cyclosporins. Iso-cyclosporins are those cyclosporins in which the linkage of the residue MeBmt at the 1-position to the residue at the 11-position is via the 3'-O-atom rather than the α-N-atom such that the MeBmt residue at position 1 has the structure of formula III:



(III)

in which -x-y- is -CH=CH- (trans).

Also included in the class are derivatised cyclosporins; cyclosporins in which the MeBmt residue is present in isomeric form (e.g. in which the configuration across positions 6' and 7' of the MeBmt residue is cis rather than trans); and cyclosporins wherein variant amino acids

are incorporated at specific positions within the peptide sequence employing e.g. the total synthetic method for the production of cyclosporins developed by R Wenger - see e.g. Traber et al 1, Traber et al, 2 and Kobel et al, loc. cit.; US Patents Nos 4,108,985; 4,220,641; 4,288, 431; 4,554,351, 4,396,542 and 4,798,823 European Patent Publications Nos. 0,034,567, 0,056,782, 0,300,784 and 0,300,785; International Patent Publication No WO 86/02080 and UK Patent Publication Nos. 2,206,119 and 2,207,678; Wenger 1, Transpl. Proc, 15 Suppl. 1:2230 (1983); Wenger 2, Angew. Chem. Int. Ed. 24 77 (1985) and Wenger 3, Progress in the Chemistry of Organic Natural Products 50 123 (1986).

The class comprised by the cyclosporins is thus now very large indeed and includes, for example, [Thr]^2-, [Val]^2-, [Nva]^2- and [Nva]^2-[Nva]^5-Ciclosporin (also known as cyclosporins C, D, G and M respectively), [Dihydro-MeBmt]^1-[Val]^2-Ciclosporin (also known as dihydro-cyclosporin D), [(D)Ser]^8-Ciclosporin, [MeIle]^{11}-Ciclosporin, [(D)MeVal]^{11}-Ciclosporin (also known as cyclosporin H), [MeAla]^6-Ciclosporin, [(D)Pro]^3-Ciclosporin and so on.

In accordance with conventional nomenclature for cyclosporins, these are defined throughout the present specification and claims by reference to the structure of Ciclosporin (i.e. Cyclosporin A). This is done by first indicating those residues in the molecule which differ from those present in Ciclosporin and then applying the term "Ciclosporin" to characterise the remaining residues which are identical to those present in Ciclosporin. At the same time the prefix "dihydro" is employed to designate cyclosporins wherein the MeBmt residue is hydrogenated (dihydro-MeBmt) i.e. wherein -x-y- in formula II is $-CH_2-CH_2-$. The prefix "iso" is employed to designate cyclosporins where the residue at the 1-position is a residue of formula III, above (iso-MeBmt) and the prefix "iso-dihydro" is used to designate cyclosporins in which the residue at the 1-position is a residue of formula III above wherein -x-y- is $-CH_2-CH_2-$ (iso-dihydro-MeBmt). Thus [Thr]^2-Ciclosporin is the cyclosporin having the sequence shown in Formula I but in which αAbu at the 2-position is replaced by Thr, and [dihydro-MeBmt]^1-[Val]^2-Ciclosporin is the cyclosporin having the sequence shown in Formula I but

in which the MeBmt residue at position 1 is hydrogenated and αAbu at the 2-position is replaced by Val. [Iso-dihydro-MeBmt]^1-[Val]^2-Ciclosporin is the cyclosporin having the sequence shown in formula I, but in which the residue at the 1-position is a residue of formula III wherein -x-y- is $-CH_2-CH_2-$ and αAbu at the 2-position is replaced by Val.

In addition amino acid residues referred to by abbreviation, e.g. Ala, MeVal, αAbu etc. are, in accordance with conventional practice, to be understood as having the (L)-configuration unless otherwise indicated, e.g. as in the case of "(D)Ala". Residue abbreviations preceded by "Me" as in the case of "MeLeu", represent α-N-methylated residues. Individual residues of the cyclosporin molecule are numbered, as in the art, clockwise and starting with the residue MeBmt or dihydro-MeBmt in position 1. The same numerical sequence is employed throughout the present specification and claims.

Some iso-cyclosporins, e.g., iso-cyclosporin A and certain 2-position derivatives (iso-cyclosporins B, D, and G), are known and have been suggested for use as pharmaceuticals. These compounds, however, have never been developed commercially as pharmaceuticals. It has been found that they are unstable; at elevated temperatures or in storage over time, they will convert from the iso-form to the cyclosporin form. Furthermore, the iso-cyclosporins were not previously thought to have any significant advantage over cyclosporins.

The problem of producing stable dosage forms for iso-cyclosporins has been solved as described herein. It has now surprisingly been found that acid addition salts of iso-cyclosporins in solid form are exceptionally stable and possess advantageous properties over the free iso-cyclosporins in solid or dissolved form. Although it is known that in acid methanolic solution cyclosporins isomerize to the corresponding iso-cyclosporins, some of which have been isolated following neutralization [Rüegger et al, Helv. Chim. Acta, 59, 1075 (1976)], and an acid addition salt of the iso-cyclosporin may be present in such solutions, no such salts have been reported to have been purified or

obtained in solid form, nor have their pharmaceutical advantages been suggested or recognized.

It has moreover surprisingly been discovered that iso-cyclosporins acid addition salts of the invention present an improved pharmacokinetic profile over cyclosporins, particularly for oral administration. One difficulty with cyclosporins is that following oral administration, the blood concentration level rapidly reaches a high peak, which is followed by a rapid drop to a low trough. Moreover, absorbtion is somewhat variable, depending on the individual and on his activity and eating patterns. The result is that oral administration of effective amounts of the cyclosporin, i.e., amounts sufficient to obtain an effective concentration at the trough level, may lead to transient but dangerously high concentrations of cyclosporin in the blood at the peak level, resulting in various undesirable side effects, particularly kidney and liver damage. It has been found the iso-cyclosporins salts of the present invention exhibit lower toxicity. It is hypothesized that the reason for this lower toxicity is that iso-cyclosporins are absorbed from the gut in the iso-form, which is relatively inactive and nontoxic, and are then subsequently converted to the pharmacologically active cyclosporin form, thus reducing the peak concentrations in the blood following administration to safer levels while maintaining a relatively constant level of active cyclosporin in the blood.

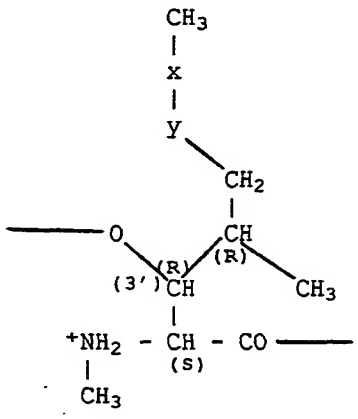
The present invention thus provides a pharmaceutically acceptable acid addition salt of an iso-cyclosporin in solid form. The invention also provides pharmaceutical compositions comprising an acid addition salt of an iso-cyclosporin in association with a pharmacologically acceptable diluent or carrier. Such compositions may comprise the acid addition salt in solid form or as a solution or suspension in a pharmaceutically acceptable solvent or diluent.

The acid addition salts of the invention are to be understood as being products in which salt formation occurs at the α-N atom of the residue at the 1-position. The term iso-cyclosporin as applied herein to the compounds of the invention is to be understood as including both

regular iso-cyclosporins e.g. where the residue at the 1-position is a residue of formula III above in which -x-y- is -CH=CH- (trans), as well as cyclosporins where the residue at the 1-position comprises the iso form of a MeBmt isomer, analogue or the like, for example iso-dihydro-cyclosporins, where the residue at the 1-position is a residue of formula III above in which -x-y- is $-CH_2-CH_2-$.

The iso-cyclosporin acid addition salts of the invention are sometimes identified herein by giving the name of the iso-cyclosporin base, followed by the formula of the associated acid. For example, [iso-MeBmt]-Ciclosporin·HCl is the hydrochloric acid addition salt of iso-cyclosporin A. In all of the examples herein, the molar ratio of acid to base present in the salt is 1:1.

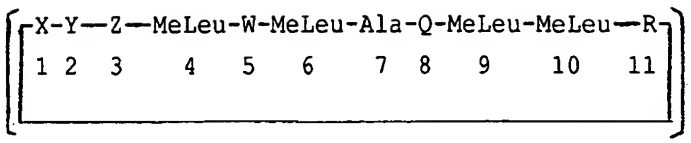
A particularly preferred embodiment is an iso-cyclosporin acid addition salt in solid form wherein the residue at the 1-position is a residue of formula IV:



(IV)

wherein -x-y- is -CH=CH- (trans) or $-CH_2-CH_2-$ and the positive charge at the nitrogen atom is satisfied by a pharmaceutically acceptable anionic species.

Especially preferred iso-cyclosporin acid addition salts in accordance with the present invention are those of formula V



(V)

wherein:

X is a residue of formula IV above,
Y is αAbu, Thr, Val or Nva,
Z is Sar, or is an α-N-methyl-(D)-α-amino acid residue, e.g., (D)MeAla, (D)MeLeu, or α-(methylthio)-(D)Sar,
W is Val or, when Y is Nva, also optionally Nva,
Q is (D)Ala, a β-hydroxy-(D)-α-amino acid residue or an ether or ester derivative of a β-hydroxy-(D)-α-amino acid residue;
R is (D)MeVal or MeVal;and
A is a pharmaceutically acceptable anion of valency b.

b may be 1 or 2, preferably 1.

A may be any pharmaceutically acceptable anion, such as, for example, Cl^-, $CH_3SO_3^-$, HSO_4^-, $H_2PO_4^-$, hydrogen maleate or pyruvate. Preferably the anion is the anion of an acid which has a pK value of <3. HSO_4^-, $H_2PO_4^-$, and especially Cl^- are particularly preferred anions.

Suitable β-hydroxy-(D)-α-amino acid residues as Q are, for example, (D)Ser or (D)Thr. Cyclosporins of this type are described and claimed in European Patent No. 056 782. Suitable ethers of β-hydroxy-(D)-α-amino acid residues include O-(2-hydroxyethyl)-(D)Ser, described and claimed in European Patent Application 414 632. Suitable esters of β-hydroxy-(D)-α-amino acid residues are described and claimed in British Patent 2 155 936. Suitable α-N-methyl-(D)-α-amino acid residues as Z include those described in European Patent 194 972.

The present invention also provides a process for the production of a pharmaceutically acceptable acid addition salt of an iso-cyclosporin in solid form, which comprises salifying an iso-cyclosporin, e.g. wherein the residue at the 1-position is iso-MeBmt or iso-dihydro-MeBmt, e.g. an iso-cyclosporin of formula V as illustrated above.

The above process step may be carried out according to conventional salification procedures, e.g. by contacting the desired iso-cyclosporin base with an appropriate acid in the presence of an inert solvent or diluent, followed by isolation of the salt in solid form. Preferably the acid is a strong acid, e.g. hydrochloric acid. The reaction is suitably performed at temperatures of from 0° to 40°C, preferably from 15° to 25°C. Isolation of the solid form may for example be by precipitation followed by separation from the liquid phase by filtration, centrifugation, etc., or by evaporation of the liquid phase, or by a combination of these methods, e.g. first concentrating the solution by evaporation, then filtering off the liquid phase.

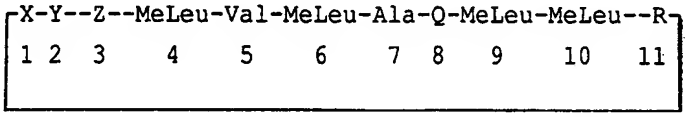
Starting materials for the above process step are known [for example, iso-cyclosporin A and processes for its production are described in Rüegger et al, loc. cit., and iso-cyclosporins D and G are known and, along with processes for their production are described in UK Patent Nos. 1,591,933 and 2,033,398 respectively], or may be prepared analogously, for example by treatment of regular cyclosporins with a strong organic acid, e.g. trifluoroacetic acid, methane sulphonic acid or p-toluenesulphonic acid (of which trifluoroacetic acid is preferred), in the presence of a solvent such as toluene, methanol, chloroform or dioxane followed by isolation of the pure base iso-cyclosporin. Reaction is suitably performed at from room temperature to 60°C, preferably at room temperature (approx. 20°C).

The iso-cyclosporin salts of the invention are characterized by proton NMR spectra showing a series of sharply defined singlet peaks in the region between 2.5 and 3.5 ppm. This indicates that they have a single well defined conformation in which the N-methylammonium group in the [iso-MeBmt]^1 residue is bridged with the carbonyl group of the

[MeLeu]^{10} residue. By contrast the NMR spectra for the iso-cyclosporin free bases is less sharply defined, with and more numerous peaks, suggesting a mixture of conformations.

The conformation of the salt form is highly stable over time and at elevated temperatures. For example, experiments measuring conversion from the iso-cyclosporin form to the cyclosporin form as shown by HPLC analysis following storage over time show that iso-cyclosporin salt forms are stable in ethanol solution indefinitely, whereas the iso-cyclosporin free base forms convert to the cyclosporin form at room temperature in ethanol solution (approximately 10% conversion after 60 hours, >50% conversion after several weeks). Even when stored at cold temperatures, e.g., in a refrigerator, the iso-cyclosporins convert to the cyclosporin form (e.g., >50% conversion after 60 days). Similarly, the iso-cyclosporin salts of the invention are stable in ethanol solution at elevated temperatures (e.g. 50°C), whereas there is substantial conversion of the free base iso-cyclosporin to cyclosporin upon mild heating in ethanol solution (e.g., approximately 70% conversion after 2 hours at 50°C). This is important because conventional methods of manufacturing tablets generally involve dissoving the components in a solvent such as ethanol and heating under evaporation conditions to obtain a solid; iso-cylosporins are not stable under these conditions, whereas the iso-cyclosporin salts of the invention are stable.

Iso-cyclosporins of formula VI:



VI

wherein X is iso-MeBmt or iso-dihydro-MeBmt;
Y is αAbu, Thr, Val or Nva;
Z is Sar, or an α-N-methyl-(D)-α-amino acid residue, e.g., (D)MeAla, (D)MeLeu, or α-(methylthio)-(D)Sar;
Q is (D)Ala or a β-hydroxy-(D)-α-amino acid residue or an

ether or ester derivative thereof, e.g. (D)Ser, (D)Thr, or O-(2-hydroxyethyl)-(D)Ser; and

R is MeVal or (D)MeVal;

provided that when Z is Sar, then Q is other than (D)Ala or R is (D)MeVal or Y is Thr,

are also new and form part of the present invention as novel products. These iso-cyclosporins have pharmaceutical utility, e.g. as immunosuppressive and anti-inflammatory agents, e.g. as may be evidenced by activity in the test methods hereinafter described, and are thus useful as pharmaceutical agents in their own right, as well as as intermediates for the production of the acid addition salts of iso-cyclosporins of the invention.

The following examples, in which all temperatures are in degrees centigrade, are illustrative of the process and methods of the present invention.

EXAMPLE 1: [Iso-MeBmt]^1-[(D)Ser]^8-Ciclosporin·HCl

a) 32.8 g of [(D)Ser]^8-Ciclosporin (27 mM) is mixed with 300 ml toluene and 10.3 ml (15.4 g) of trifluoroacetic acid and allowed to stand for 66 hr at room temperature. Then 300 ml ethyl acetate is added and the solution is washed in a separating funnel with 300 ml saturated $NaHCO_3$ solution. The organic phase is dried over sodium sulphate, filtered and the solvent is evaporated. The free base is purified by recrystallization from isopropyl ether/ methylene chloride. M.p. 142-146°

b) 10 Grams of [iso-MeBmt]^1-[(D)Ser]^8-Ciclosporin free base are dissolved in 10 ml ethyl acetate. Then 300 ml ethyl ether is added and HCl gas is passed into the solution until pH test paper shows pH 2. The title compound (hydrochloric acid addition salt) precipitates out.

m.p. 170-172°C; $[\alpha]_D^{20}$ = -199.7° c = 1.0 in $CHCl_3$

EXAMPLE 2: [Iso-MeBmt]^1-[Thr]^2-Ciclosporin·HCl

12 g [Iso-MeBmt]^1-[Thr]^2-Ciclosporin is dissolved in 100 ml diethyl-ether and adjusted to pH 3.0 by addition of HCl in diethyl ether whereupon the title compound precipitates out.

m.p. 173-175°C; $[\alpha]_D^{20}$ = -223°; c = 0.91 in $CHCl_3$.

EXAMPLE 3: [Iso-MeBmt]^1-Ciclosporin·HCl

The title compound is produced by analogy with Example 2, using iso-cyclosporin A as starting material.

$[\alpha]_D^{20}$ = -187.9° ; c = 0.98 in $CHCl_3$

EXAMPLE 4: [Iso-MeBmt]^1-[O-(2-hydroxyethyl)(D)Ser]^8-Ciclosporin·HCl

a) 10.0 g [O-(2-hydroxyethyl)(D)Ser]^8-Ciclosporin is dissolved in 100 ml toluene and treated at 20°C with 3.03ml trifluoroacetic acid. The mixture is stirred at 20°C for 65 hr and the oil which separates out is dissolved by addition of 200 ml ethyl acetate. The organic phase is washed in a separating funnel with 50 ml saturated sodium bicarbonate solution, and then dried, filtered and evaporated. The residue is purified by chromatography on silica gel with ethyl acetate / methanol 92:8 and recrystallized from diethyl ether to give [iso-MeBmt]^1-[O-(2-hydroxyethyl)(D)Ser]^8-Ciclosporin free base.

b) 5.5 g of the above base form is dissolved in 10 ml ethyl acetate plus 10 ml ether and precipitated by the addition of a saturated solution of HCl in ether to give the hydrochloric acid addition salt form, which is separated by filtration.

$[\alpha]_D^{20}$ = -178°; c = 1.0 in $CHCl_3$

EXAMPLE 5: [Iso-MeBmt]^1-[Nva]^2-Ciclosporin·HCl

The title compound (hydrochloric acid addition salt) is prepared by analogy to example 2 using iso-cyclosporin G as starting material.

$[\alpha]_D^{20}$ = -185°; c = 0.9 in $CCCl_3$

EXAMPLE 6: [Iso-dihydroMeBmt]^1-[Val]^2-[α-(methylthio)-(D)Sar]^3-Ciclosporin·HCl

The free base form of the title compound is prepared by analogy to example 4a using [dihydroMeBmt]^1-[Val]^2-[α-(methylthio)(D)Sar]^3-Ciclosporin (prepared, e.g., as described in European patent 0 194 972, example 21) as the starting material.

The title compound as hydrochloric acid addition salt is prepared by analogy to example 4b.

$[\alpha]_D^{20}$ = -168°; c = 0.9 in $CHCl_3$

EXAMPLE 7: [Iso-dihydroMeBmt]^1-[α-(methylthio)-(D)Sar]^3-Ciclosporin·HCl

The free base form of the title compound is prepared by analogy to example 4a using [dihydroMeBmt]^1-[α-(methylthio)(D)Sar]^3- Ciclosporin (prepared, e.g., as described in European patent 0 194 972, example 23) as the starting material.

The title compound as hydrochloric acid addition salt is prepared by analogy to example 4b.

$[\alpha]_D^{20}$ = -168°; c = 0.9 in $CHCl_3$

EXAMPLE 8: [Iso-MeBmt]^1-[(D)MeVal]^{11}-Ciclosporin·HCl

The free base form of the title compound is prepared by analogy to example 4a using cyclosporin H as the starting material.

The title compound as hydrochloric acid addition salt is prepared by analogy to example 4b.

$[\alpha]_D^{20}$ = -167°; c = 0.88 in $CHCl_3$

EXAMPLE 9: [Iso-MeBmt]^1-[Val]^2-Ciclosporin·HCl

The title compound as hydrochloric acid addition salt is prepared by analogy to example 2 using iso-cyclosporin D as starting material.

m.p. 171-174°C; $[\alpha]_D^{20}$ = -201°; c = 0.94 in $CHCl_3$

EXAMPLE 10: [Iso-MeBmt]^1-Ciclosporin·H_2SO_4

12 g [Iso-MeBmt]^1-Ciclosporin is dissolved in 100 ml diethylether and an equimolar amount of H_2SO_4 is added, whereupon the title compound

(sulfuric acid addition salt) precipitates out and is removed by filtration.

m.p. 152-172°C; $[\alpha]_D^{20} = -204°$; c = 0.95 in $CHCl_3$.

EXAMPLE 11: [Iso-MeBmt]^1-Ciclosporin·H_3PO_4

12 g [Iso-MeBmt]^1-Ciclosporin is dissolved in 100 ml diethylether and an equimolar amount of H_3PO_4 is added, whereupon the title compound (phosphoric acid addition salt) precipitates out and is removed by filtration.

m.p. 139-148°C; $[\alpha]_D^{20} = -185°$; c = 0.95 in $CHCl_3$.

EXAMPLE 12: [Iso-MeBmt]^1[(D)Ser]^8-Ciclosporin·H_2SO_4

12 g [Iso-MeBmt]^1-[(D)Ser]^8-Ciclosporin prepared as in example 1 is dissolved in 100 ml diethylether and an equimolar amount of H_2SO_4 added, whereupon the title compound (sulfuric acid addition salt) precipitates out and is removed by filtration.

m.p. 154-162°C: $[\alpha]_D^{20} = -173°$; c = 0.93 in $CHCl_3$.

EXAMPLE 13: [Iso-MeBmt]^1-[(D)Ser]^8-Ciclosporin·H_3PO_4

12 g [Iso-MeBmt]^1-[(D)Ser]^8-Ciclosporin prepared as in example 1 is dissolved in 100 ml diethylether and an equimolar amount of H_3PO_4 is added, whereupon the title compound (phosphoric acid addition salt) precipitates out and is removed by filtration.

m.p. 154-162°C; $[\alpha]_D^{20} = -173°$; c = 0.93 in $CHCl_3$.

The iso-cyclosporin acid addition salts of the present invention, in particular those of formula V, as well as the novel iso-cyclosporins of Formula VI (all hereinafter collectively referred to as "Product Iso-cyclosporins") possess pharmaceutical utility. Their activity in

vitro is in part a function of the length of the test; in shorter tests, such as the cyclophilin binding assay, they appear less active, but in longer in vitro tests for immunosuppression in cell cultures, such as the mixed lymphocyte reaction, when the compounds are exposed to physiologic conditions for substantial period of time, the compounds exemplified herein exhibit activity on the same order of magnitude as cyclosporin A. (For a description of test methods for in vitro evaluation of cyclosporins, see, e.g., Baumann, et al. Transplantation Proc. 24 (1992) 43). The reason for this is that the Product Iso-cyclosporins act as pro-drugs which slowly convert under physiologic conditions to the normal cyclosporin forms. This conversion can also be shown by HPLC measurements of the levels of the iso-cyclosporin / cyclosporin in the blood following administration of the compounds. As discussed above, this slow conversion from the inactive form to the active form is a high advantage as it can be shown to decrease the peak concentration of active cyclosporin in the blood and to provide a sustained release over time, thereby reducing toxic peak concentration levels while nevertheless maintaining pharmacologically active concentrations in the blood.

The pharmaceutical utility of the Product Iso-cyclosporins is demonstrated, e.g. in the following test methods:

1) Localised Graft-Versus-Host (GvH) Reaction [Ford et al, TRANSPLANTATION 10 (1970) 258]

Spleen cells (1×10^7) from 6 week old female Wistar/Furth (WF) rats are injected subcutaneously on day 0 into the left hind-paw of female (F344 x WF)F_1 rats weighing about 100g. Animals are treated for 4 consecutive days and the popliteal lymph nodes are removed and weighed on day 7. The difference in weight between the two lymph nodes is taken as the parameter for evaluating the reaction. Product Iso-cyclosporins are active in the above test method at dosages of, e.g. 25 mg/kg to 35 mg/kg p.o. and 10 mg/kg to 15 mg/kg s.c.

2) Kidney Allograft Reaction in Rat

One kidney from a female fisher 344 rat is transplanted onto the renal vessel of a unilaterally (left side) nephrectomised WF recipient rat using an end-to-end anastomosis. Ureteric anastamosis is also end-to-end. Treatment commences on the day of transplantation and is continued for 14 days. A contralateral nephrectomy is done seven days after transplantation, leaving the recipient relying on the performance of the donor kidney. Survival of the graft is taken as the parameter for a functional graft. Product Iso-cyclosporins are active in the above test method at dosages of e.g. 5 mg/kg to 7.5 mg/kg p.o.

3) Experimentally Induced Allergic Encephalomyelitis (EAE) in Rats [Levine & Wenk, AMER J PATH 47 (1965) 61; McFarlin et al, J IMMUNOL 113 (1974) 712; Borel, TRANSPLANT. & CLIN. IMMUNOL 13 (1981) 3]

Male Wistar rats are injected in the hind paws with a mixture of bovine spinal cord and complete Freund's adjuvant. Symptoms of the disease (paralysis of the tail and both hind legs) usually develop within 16 days. The number of diseased animals as well as the time of onset of the disease are recorded. Product Iso-cyclosporins are active in the above test method at dosages of, e.g. 25 mg/kg to 30 mg/kg p.o.

4) Freund's Adjuvant Arthritis [Winter & Nuss, ARTHRITIS & RHEUMATISM 9 (1966) 394; Billingham & Davies, HANDBOOK OF EXPERIMENTAL PHARMACOL (Vane & Ferreira Eds, Springer-Verlag, Berlin) 50/II (1979) 108-144]

OFA and Wistar rats (male or female, 150g body weight) are injected i.c. at the base of the tail or in the hind paw with 0.1 ml of mineral oil containing 0.6 mg of lyophilised heat-killed Mycobacterium smegmatis. In the developing arthritis model, treatment is started immediately after the injection of the adjuvant (days 1 - 18); in the established arthritis model treatment is started on day 14, when the secondary inflammation is well developed (days 14-20). At the end of the experiment, the swelling of the joints is measured by means of a microcaliper. ED_{50} is the oral dose in mg/kg which reduces the swelling

(primary or secondary) to half of that of the controls. Product Iso-cyclosporins are active in the above test method at dosages of, e.g. 15 to 25 mg/kg p.o.

Product Iso-cyclosporins are therefore useful as pharmaceuticals, e.g. as immunosuppressive as well as anti-inflammatory agents.

Product Iso-cyclosporins are particularly useful for the prevention of organ or tissue transplant rejection, e.g. for the treatment of recipients of e.g. heart, lung, combined heart-lung, liver, kidney, pancreatic, skin or corneal transplants. They are also indicated for the prevention of graft-versus-host disease, such as following bone marrow transplantation.

Product Iso-cyclosporins are also useful for the treatment of autoimmune disease and of inflammatory conditions, in particular inflammatory conditions with an aetiology including an autoimmune component such as arthritis (for example rheumatoid arthritis, arthritis chronica progrediente and arthritis deformans) and rheumatic diseases. Specific autoimmune diseases for which the cyclosporins of the invention may be employed include, autoimmune haematological disorders (including e.g. haemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopaenia), systemic lupus erythematosus, polychondritis, sclerodoma, Wegener granulamatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (including e.g. ulcerative colitis and Crohn's disease) endocrine ophthalmopathy, Graves disease, sarcoidosis, multiple sclerosis, primary billiary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal change nephropathy) and juvenile dermatomyositis.

Product Iso-cyclosporins are further indicated for use in the treatment of other diseases or conditions for which cyclosporins, e.g. Ciclosporin, therapy is practised or proposed, for example, for the treatment of alopecia/the promotion of hair growth and for the treatment of asthma, e.g. on administration by inhalation. They are also indicated for use as anti-parasitic agents, in particular for the treatment of parasitic, e.g. protozoal, fungal or vermicular infection or invasion, for example in the treatment of filariasis, schistosomiasis, coccidiomycosis or plasmoidal infection, e.g. malaria. They are yet further indicated for use in the reversal of resistance of malignancies or infections to other chemotherapy (multidrug resistance), as well as the enhancement of wound healing.

For these indications, the appropriate dosage will, of course, vary depending upon, for example the Product Iso-cyclosporin employed, the host, the mode of administration and the nature and severity of the condition being treated. With organ transplant, for example, the dosage will vary during the course of the treatment. However, in general, satisfactory results are indicated to be obtained at daily dosages of from about 2.5 to 15 mg/kg, preferably from about 2.5 to 7.5 mg/kg conveniently administered in divided doses of from, e.g. 800 mg preferably 400 mg or in sustained release form. The Product Iso-cyclosporins may be administered by any conventional route, in particular enterally, e.g. in the form of solutions for drinking, or in tablet or capsule form, or parenterally e.g. in the form of injectable solutions or suspensions. Conventional galenic formulations into which the Product Iso-cyclosporins may be incorporated are described, e.g. in Remington's Pharmaceutical Sciences (Mack Publishing Co. , Easton PA).

The iso-cyclosporin salts of the invention are characterised by improved stability as compared with known iso-cyclosporins, which enables them to be more readily incorporated into stable solid galenic forms. They also possess modified solubility characteristics.

These and other physical properties render the iso-cyclosporin salts surprisingly advantageous in terms of both the range of galenic forms

which can be prepared employing the compounds and the bioavailability characteristics thereby achievable. This finding is of material significance and benefit over the known difficulties hitherto encountered in the art and over the properties, such as the hydrophobicity, inherent to known cyclosporins. The hydrophobic/lipophilic character of known cyclosporins has severely limited the range of galenic forms in which cyclosporins may be used, allowing formulation in a liquid fatty state only, for example encapsulation in soft gelatin capsules. The enhanced solubility and stability properties of the iso-cyclosporin salts of the invention permit provision of solid dosage forms such as powders, granulates and tablets, which contain the product cyclosporins in concentrations sufficiently high to permit convenient use and yet also meet the required criteria in terms of bioavailability, e.g. enabling effective resorption from the stomach or gut lumen and achievement of consistent and appropriately high blood/blood serum levels.

A suitable tablet formulation for the iso-cyclosporin salts of the invention comprises the following excipients:

a) iso-cyclosporin salt as active ingredient
b) a saccharide or fatty acid saccharide monoester
c) a solid, e.g. polymeric carrier
d) a water swellable or water soluble component; and
e) a binder or lubricant.

More specifically, an example of the ingredients for the tablet formulation is formulation A:

INGREDIENT	RELATIVE AMOUNT (mg)
a) Product Iso-cyclosporin (salt form)	50.0
b) Saccharide monolaurate L-1695*	300.0
c) Crospovidone (Polyplasdone XL)	25.0
d^1) Hydroxypropylmethylcellulose (Pharmacoate 603)	25.0
d^2) Cellulose (Avicel PH 101)	200.0
e) Sodiumlauryl sulphate	100.0
TOTAL	700.0

[* Product commerically available from Mistubishi-Kasei Food Corp., Tokyo 104, Japan: HLB-value = at least 12.3; lauryl ester residue purity = at least 95%: M.P. = ca. 35°C: decomposition at ca. 235°C: surface tension of 0.1% by weight aqueous solution = ca. 72.0 dyn/cm at 25°C.]

An alternative and preferred tablet formulation B contains the following ingredients:

INGREDIENT	RELATIVE AMOUNT (mg)
a) Product Iso-cyclosporin (salt form)	50.0
b) Lactose DC 21 DMV	68.5
c) Crospovidone (Polyplasdone XL)	30.0
d^1) Hydroxypropylmethylcellulose 3 cps	100.0
d^2) Cellulose microcrystalline	70.0
e^1) Sodiumlauryl sulphate	5.0
e^2) Magnesium stearate	1.5
TOTAL	325.0

In the manufacture of formulation B, the drug and hydroxypropyl-methyl cellulose are dissolved in a mixture of absolute ethanol and acetone. The solvents are evaporated in a rotovapor, and the solid dispersion is milled in a ball mill, then mixed with the other components and compressed into tablets.

In accordance with the foregoing the present invention also provides in a further series of embodiments:

A. A method of effecting immunosuppression in a subject in need of such treatment which method comprises administering to said subject an effective amount of a Product Iso-cyclosporin.

B. A method:

i) for the prevention of organ transplant rejection, for example for the treatment of recipients of organ transplants of any of the particular types hereinbefore set forth, or graft-vs-host disease; or

ii) for the treatment of autoimmune disease or for the treatment of inflammatory conditions, for example for the treatment of any such disease or condition hereinbefore set forth; or

iii) for the treatment of alopecia or for the promotion of hair growth; or

iv) for the treatment of parasitic infection or invasion, for example, for the treatment of protozoal, fungal or vermicular infection or invasion, e.g. for the treatment of coccidiomycosis or malaria; or

v) for the reversal of reduced resistance of malignancies or infections to chemotherapy (multidrug resistance) or for the enhancement of wound healing; or

vi) for the treatment of asthma;

in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a Product Iso-cyclosporin.

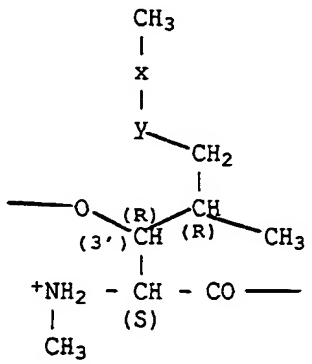
C. A Product Iso-cyclosporin for use as a pharmaceutical, e.g. for use as an immunosuppressant or for use in the treatment of any disease or condition as set forth under B above.

D. A pharmaceutical composition comprising a Product Iso-cyclosporin together with a pharmaceutically acceptable diluent or carrier therefor.

CLAIMS

1. A pharmaceutically acceptable acid addition salt of an iso-cyclosporin in solid form.

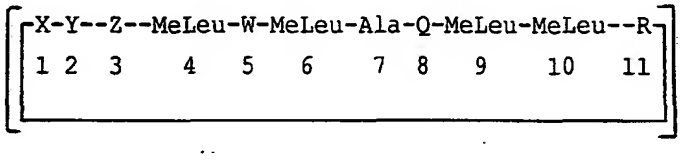
2. An iso-cyclosporin salt according to claim 1 wherein the residue at the 1-position is a residue of formula IV



(IV)

wherein -x-y- is -CH=CH- (trans) or $-CH_2-CH_2-$ and the positive charge at the nitrogen atom is satisfied by a pharmaceutically acceptable anionic species.

3. An iso-cyclosporin salt according to claim 2 of formula V



(V)

wherein: X is a residue of formula IV as illustrated in claim 2
Y is αAbu, Thr, Val or Nva;
Z is Sar, or an α-N-methyl-α-(D)amino acid residue;
W is Val or, when Y is Nva, optionally also Nva;
Q is (D)Ala, a β-hydroxy-(D)-α-amino acid or an ether or ester derivative thereof;
R is MeVal or (D)MeVal; and

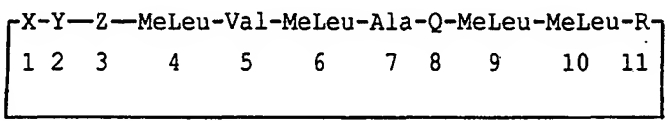
A is a pharmaceutically acceptable anion of valency b, wherein b is 1 or 2.

4. An iso-cyclosporin salt according to claim 3 wherein:
Z is Sar, (D)MeAla, (D)MeLeu, or α-(methylthio)-(D)Sar;
W is Val;
Q is (D)Ala, (D)Thr, (D)Ser, or O-(2-hydroxyethyl)-(D)Ser;
A is Cl^-, HSO_4^-, or $H_2PO_4^-$; and
b is 1.

5. An isocyclosporin salt according to claim 4 selected from the following:

i) [Iso-MeBmt]^1-[(D)Ser]^8-Ciclosporin·HCl,
ii) [Iso-MeBmt]^1-[Thr]^2-Ciclosporin·HCl,
iii) [Iso-MeBmt]^1-Ciclosporin·HCl,
iv) [Iso-MeBmt]^1-[O-(2-hydroxyethyl)(D)Ser]^8-Ciclosporin·HCl,
v) [Iso-MeBmt]^1-[Nva]^2-Ciclosporin·HCl,
vi) [Iso-dihydro-MeBmt]^1-[Val]^2-[α-(methylthio)-(D)Sar]^3-Ciclosporin·HCl,
vii) [Iso-dihydro-MeBmt]^1-[α-(methylthio)-(D)Sar]^3-Ciclosporin·HCl,
viii) [Iso-MeBmt]^1-[(D)MeVal]^{11}-Ciclosporin·HCl,
ix) [Iso-MeBmt]^1-[Val]^2-Ciclosporin·HCl,
x) [Iso-MeBmt]^1-Ciclosporin·H_2SO_4,
xi) [Iso-MeBmt]^1-Ciclosporin·H_3PO_4,
xii) [Iso-MeBmt]^1[(D)Ser]^8-Ciclosporin·H_2SO_4, and
xiii) [Iso-MeBmt]^1-[(D)Ser]^8-Ciclosporin·H_3PO_4.

6. An iso-cyclosporin of formula VI



(VI)

wherein: X is iso-MeBmt or iso-dihydro-MeBmt;

Y is αAbu, Thr, Val or Nva;
Z is Sar, or an α-N-methyl-α-(D)-amino acid residue;
Q is (D)Ala or a β-hydroxy-(D)-α-amino acid or an ester or ether derivative thereof; and
R is MeVal or (D)MeVal;

provided that when Z is Sar, then Q is other than (D)Ala or Y is Thr or R is (D)MeVal.

7. An iso-cyclosporin according to claim 6 wherein Z is Sar, (D)MeAla, (D)MeLeu, or α-(methylthio)-(D)Sar; and Q is (D)Ala, (D)Thr, (D)Ser, or O-(2-hydroxyethyl)-(D)Ser.

8. An iso-cyclosporin according to claim 7 selected from the following:

i) [Iso-MeBmt]^{1}-[(D)Ser]^{8}-Ciclosporin,
ii) [Iso-MeBmt]^{1}-[Thr]^{2}-Ciclosporin,
iii) [Iso-MeBmt]^{1}-[O-(2-hydroxyethyl)(D)Ser]^{8}-Ciclosporin,
iv) [Iso-dihydro-MeBmt]^{1}-[Val]^{2}-[α-(methylthio)-(D)Sar]^{3}-Ciclosporin,
v) [Iso-dihydro-MeBmt]^{1}-[α-(methylthio)-(D)Sar]^{3}-Ciclosporin, and
vi) [Iso-MeBmt]^{1}-[(D)MeVal]^{11}-Ciclosporin.

9. A pharmaceutical composition comprising an iso-cyclosporin acid addition salt in solid form or in solution or suspension in a pharmacologically acceptable solvent or diluent.

10. An iso-cyclosporin acid addition salt for use as a pharmaceutical.

11. Use of an iso-cyclosporin acid addition salt according to any one of claims 1-5 or an iso-cyclosporin according to any one of claims 6-8 in the manufacture of a medicament for prophylaxis or treatment of any one or more of the following conditions:

i) organ transplant rejection or graft-vs-host disease;

ii) autoimmune diseases or inflammatory conditions;

iii) alopecia or inadequate hair growth;

iv) parasitic infection or invasion;

v) multidrug resistance; or

vi) asthma.

12. A process for making an iso-cyclosporin acid addition salt in solid form comprising the steps of i) contacting an iso-cyclosporin with a strong acid, and ii) isolating the salt.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/00407

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/64; A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	CH,A,641 356 (SANDOZ AG) 29 February 1984 see claims 1,3,8	1-12
A	GB,A,2 033 398 (SANDOZ AG) 21 May 1980 see example 3	1-12
P,A	INT.J.PHARM. vol. 82, no. 1-2, 20 April 1992, AMSTERDAM pages 85 - 90 H.BUNDGAARD ET AL 'PRODRUGS OF PEPTIDES. 16. ISOCYCLOSPORIN A AS A POTENTIONAL PRODRUG OF CYCLOSPORIN A' see the whole document	1-12

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 JUNE 1993	[illegible]
International Searching Authority	**Signature of Authorized Officer**
EUR PEAN PATENT OFFICE	GROENENDIJK M.S.M.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9300407
SA 71022

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
CH-A-641356	29-02-84	None		
GB-A-2033398	21-05-80	CH-A-	639961	15-12-83
		CH-A-	642954	15-05-84
		CH-A-	637124	15-07-83
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		CA-A-	1129359	10-08-82
		DE-A,C	2941080	08-05-80
		FR-A,B	2439182	16-05-80
		NL-A-	7907609	22-04-80
		SE-B-	448386	16-02-87
		SE-A-	7908350	19-04-80
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